Supporting Information

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SI Materials and Methods

Strains. The WT strain is the standard N2 Bristol strain. Other strains are listed below in the order in which they appear in the figures: MT18636 *nIs326*[*gcy-33::CAMELEON*; *lin-15AB(n765)*]; HA1203 *rtIs25*[*sra-6::CAMELEON*]; FQ100 *wzIs42*[*sre-1::CAMELEON*]; *lin-15AB(n765)*; FQ94 *wzIs38*[*str-1::CAMELEON*]; *lin-15AB(n765)*; FQ136 *tax-4(p678)*; *nIs326[gcy-33::CAMELEON*]; FQ14 *tax-2(p671)*; *nIs326[gcy-33::CAMELEON*]; PS5883 *rgs-3 (ok2288)*; *nIs326[gcy-33::CAMELEON*]; PS5884 *npr-1(ad609)*; *nIs326[gcy-33::CAMELEON]*; PS6220 *daf-11(m47)*; *nIs326[gcy-33::CAMELEON*]; PS6221 *daf-2(e1370)*; *nIs326[gcy-33::CAMELEON*]; PS6222 *daf-7(e1372)*; *nIs326[gcy-33::CAMELEON*]; FQ31 *gcy-33(ok232)*; *nIs326[gcy-33::CAMELEON]*; *gcy-31 (ok296)*]; MT17370 *nIs242[gcy-33::gfp]*; *lin-15(n765)*; FX02816 *gcy-9(tm2816)*; MT14525 *gcy-9(n4470)*; PS6272 *nIs323[gcy-33::CAMELEON]*; *gcy-9(tm2816)*; PS6273 *nIs323[gcy-33::CAMELEON]*; *gcy-9(n4470)*; PS6264 *gcy-9(tm2816)*; *syEx1175[gcy-33::gcy-9*; *myo-2::dsRed]*; PS6265 *nIs323[gcy-33::CAMELEON]*; *gcy-9(tm2816)*; *syEx1175[gcy-33::gcy-9*; *myo-2::dsRed]*; CX11697 *kyIs536[flp-17::p17::s12GFP*, *elt-2::mCherry]*; *kyIs538[glb-5::p12::s12GFP*, *elt-2::mCherry]*, which contains a genetic ablation of the BAG neurons; MT18629 *nIs323[gcy-33::CAMELEON]*; *lin-15AB(n765)*; PS6229 *nIs323[gcy-33::CAMELEON]*; RB1780 *rgs-3(ok2288)*; PS5927 *rgs-3(ok2288)*; *syEx1108[gcy-33::rgs-3*; *pax-2::gfp]*; CB1372 *daf-7(e1372)*; DA2202 *daf-7(e1372)*; *adEx2202 [gpa-4::daf-7*, *rol-6::GFP]*; PS5892 *gcy-33(ok232)*; *gcy-31(ok296)*; FX02669 *gcy-1(tm2669)*; VC2796 *gcy-3(gk1154)*; FX01653 *gcy-4 (tm16530)*; FX01449 *gcy-6(tm1449)*; FX00901 *gcy-7(tm901)*; PS4054 *gcy-8(sy664)*; CX2065 *odr-1(n1936)*; IK212 *gcy-12(nj10)*; VC2440 *gcy-14(ok2236)*; VC2675 *gcy-15(gk1102)*; FX04516 *gcy-17(tm4516)*; VC2321 *gcy-18(ok3047)/nT1[qIs51]*; *+/nT1[qIs51]*; RB1909 *gcy-19(ok2472)*; RB1935 *gcy-20(ok2538)*; FX02364 *gcy-22(tm2364)*; IK597 *gcy-23(nj37) gcy-8(oy44) gcy-18(nj38)*; FX04300 *gcy-25(tm4300)*; RB2622 *gcy-27(ok3653)*; FX02411 *gcy-28(tm2411)*; RB626 *gcy-37(ok384)*; MT15933 *flp-17(n4894)*. *gcy-9(tm2816)* was used for all experiments involving *gcy-9* unless otherwise indicated.

Transgene Construction. To generate *gcy-33::CAMELEON* constructs, 1.4-kb 5′ to the *gcy-33* translational start was cloned into the expression vector pCVG6. pCVG6 is a version of the *Caenorhabditis elegans* expression vector pPD49.26 containing cameleon YC3.60 (1) and was kindly provided by Chris Gabel, Harrison Gabel, and Aravinthan Samuel (Harvard University, Cambridge, MA). ADL and AWB reporter constructs were made by cloning into pCVG6 4.0- and 3.5-kb sequences 5′ to the translational starts of *sre-1*and *str-1*, respectively. *CAMELEON* expression constructs were injected into *lin-15AB(n765)* animals at 100 ng/μL together with 50 ng/μL of the *lin-15* rescuing plasmid EKL15. Extrachromosomal arrays were integrated by γ-irradiation to create the integrated transgenes *nIs323[gcy-33::CAMELEON]*, *nIs326[gcy-33::CAMELEON]*, *wzIs42[sre-1::CAMELEON]*, and *wzIs38[str-1::CAMELEON]*.

To generate the *gcy-33::rgs-3* rescue construct, 1 kb of the *gcy-33* promoter was cloned into pHA #443, an expression vector containing *rgs-3* cDNA (2). The transgene was injected into RB1780 [*rgs-3(ok2288)*] animals at 50 ng/μL together with 50 ng/μL of *guEX64*[*pax-2::GFP]* as a coinjection marker. Three of six independent lines rescued the *rgs-3(ok2288)* CO_2 response defect.

To generate the *gcy-33::gcy-9* rescue construct, the 3,246-bp *gcy-9* cDNA was amplified from N2 animals using the following primers: 5′ primer, ATGCGTTTATATTTATTTTTCATTTCTTC; 3′ primer, TCATTGTTTGCCGGTTCTTCCTTC. The *gcy-9* cDNA was then cloned into pPD49.26 containing 1 kb of the *gcy-33* promoter. The transgene was injected into FX02816 [*gcy-9(tm2816)*] animals at 10 ng/μL together with 10 ng/μL of *myo-2::dsRed* as a coinjection marker. Of the three independent lines that were obtained, one fully rescued and one partly rescued the CO_2 avoidance defect of *gcy-9(tm2816)* mutants.

1. Nagai T, Yamada S, Tominaga T, Ichikawa M, Miyawaki A (2004) Expanded dynamic range of fluorescent indicators for $Ca^{(2+)}$ by circularly permuted yellow fluorescent proteins. *Proc Natl Acad Sci USA* 101:10554–10559.

2. Ferkey DM, et al. (2007) *C. elegans* G protein regulator RGS-3 controls sensitivity to sensory stimuli. *Neuron* 53:39–52.

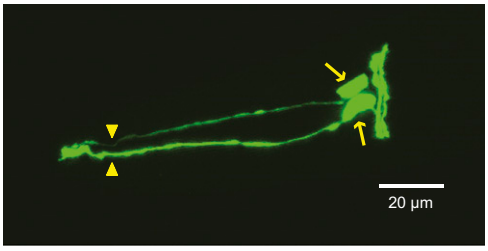

Fig. S1. BAG neurons in *C. elegans.* Maximum projection image of a series of confocal micrographs of the paired BAG neurons. BAG neuron cell bodies are located in the head and extend dendrites to the tip of the nose. Anterior is to the left. Arrows indicate cell bodies; arrowheads indicate dendrites.

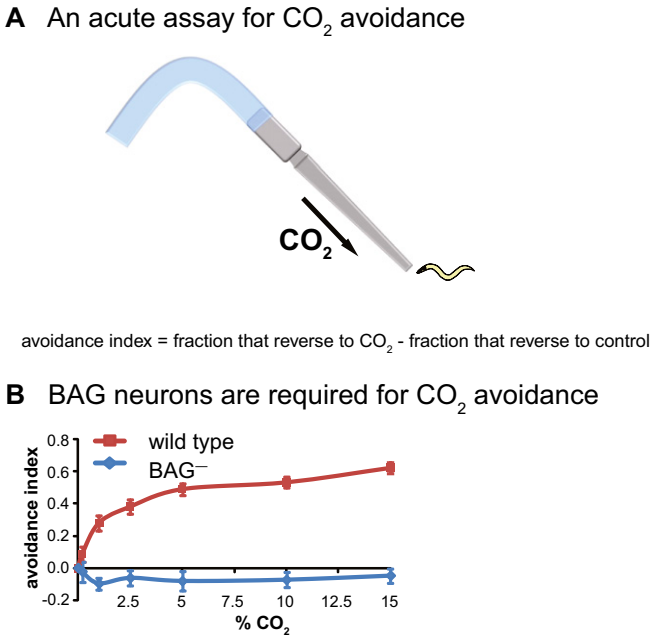

Fig. S2. BAG neurons are required for CO_2 avoidance behavior. (*A*) An assay for acute CO_2 avoidance. The head of a forward-moving worm is exposed to CO_2, and the worm is given 4 s to reverse (1). (*B*) BAG neurons are required for avoidance of a range of concentrations of CO_2. Animals that lack BAG neurons do not respond to CO_2. BAG^- animals were engineered to express a transgene that specifically kills the BAG neurons (n = 3–16 trials for BAG^- animals; data for wild-type animals are from ref. 1).

1. Hallem EA, Sternberg PW (2008) Acute carbon dioxide avoidance in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 105:8038–8043.

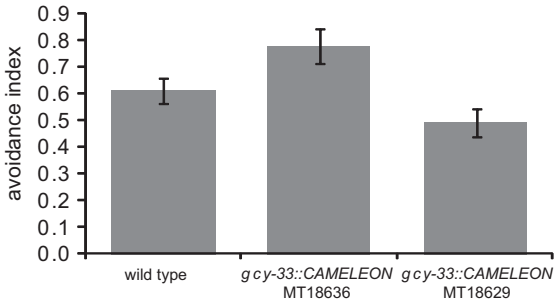

Fig. S3. Animals expressing *gcy-33::CAMELEON* respond normally to CO_2. These lines contain integrated transgenes that express cameleon YC3.60 specifically in the BAG neurons (n = 9–32 trials for each genotype).

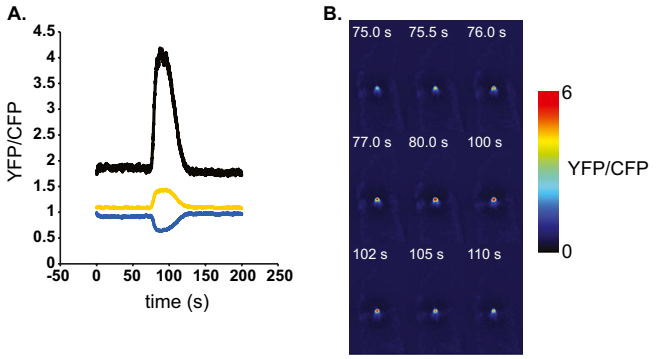

Fig. S4. BAG neurons are activated rapidly and reversibly by CO_2. (*A*) Animals were exposed to a 25-s pulse of 5% CO_2. Calcium transients were imaged using cameleon YC3.60 driven by the BAG-specific *gcy-33* promoter. Black trace depicts the YFP to CFP ratio over the entire time course of the experiment. Yellow and blue traces illustrate changes in YFP and CFP emissions, respectively (in arbitrary units). The 25-s pulse of 5% CO_2 begins at 75 s and ends at 100 s. (*B*) An image sequence from the recordings shown in *A*. Time points for each frame are indicated. The time point at 75 s shows the last frame before the CO_2 pulse, and the time point at 100 s shows the last frame before the end of the CO_2 pulse. Colors indicate the YFP to CFP ratio; the scale is linear, and red corresponds to high calcium.

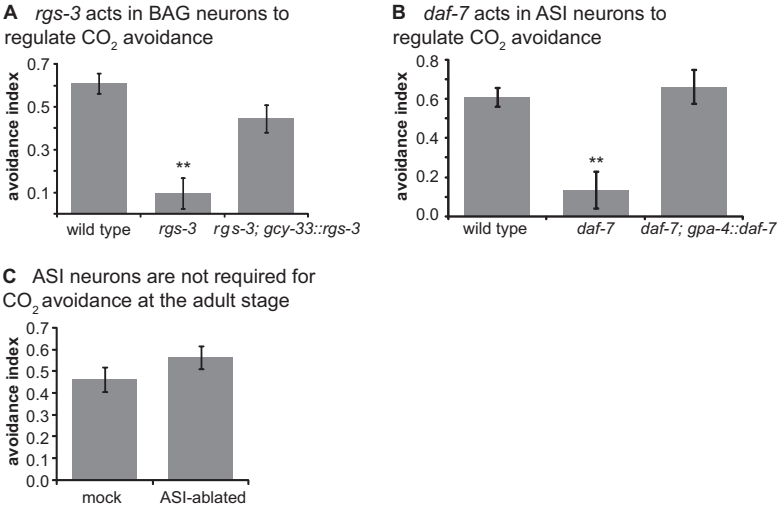

Fig. S5. *rgs-3* is required in the BAG neurons, and *daf-7* is required in the ASI neurons for CO_2 avoidance behavior. (*A*) *rgs-3* mutants do not respond to CO_2, and cell-specific rescue of *rgs-3* in the BAG neurons using the *gcy-33* promoter restores CO_2 response, suggesting that G protein signaling negatively regulates CO_2 response within the BAG neurons. (*B*) *daf-7* mutants do not respond to CO_2, and rescue of *daf-7* in the ASI neurons using the *gpa-4* promoter restores acute CO_2 avoidance (n = 10–32 trials for each genotype). (*C*) Ablation of ASI neurons does not affect CO_2 avoidance behavior, showing that ASI neurons are not required for CO_2 detection (n = 11–22 animals). For all graphs, error bars represent SEMs.

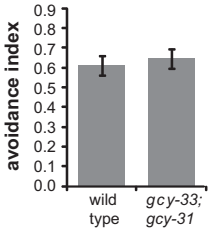

Fig. S6. *gcy-33; gcy-31* double mutants respond normally to CO_2 (n = 12–32 trials for each genotype).

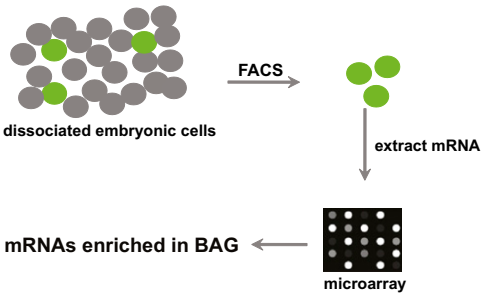

Fig. S7. Transcriptional profiling of embryonic BAG neurons. Embryos containing fluorescently labeled BAG neurons expressing a *gcy-33::GFP* transgene were dissociated with chitinase and sorted by FACS (1) to isolate BAG neurons. mRNA was extracted from BAG neurons and used to generate cDNA, which was hybridized to a *C. elegans* microarray. Transcripts significantly enriched in BAG relative to all embryonic cells were then identified as described in *Materials and Methods*.

1. Christensen M, et al. (2002) A primary culture system for functional analysis of *C. elegans* neurons and muscle cells. *Neuron* 33:503–514.

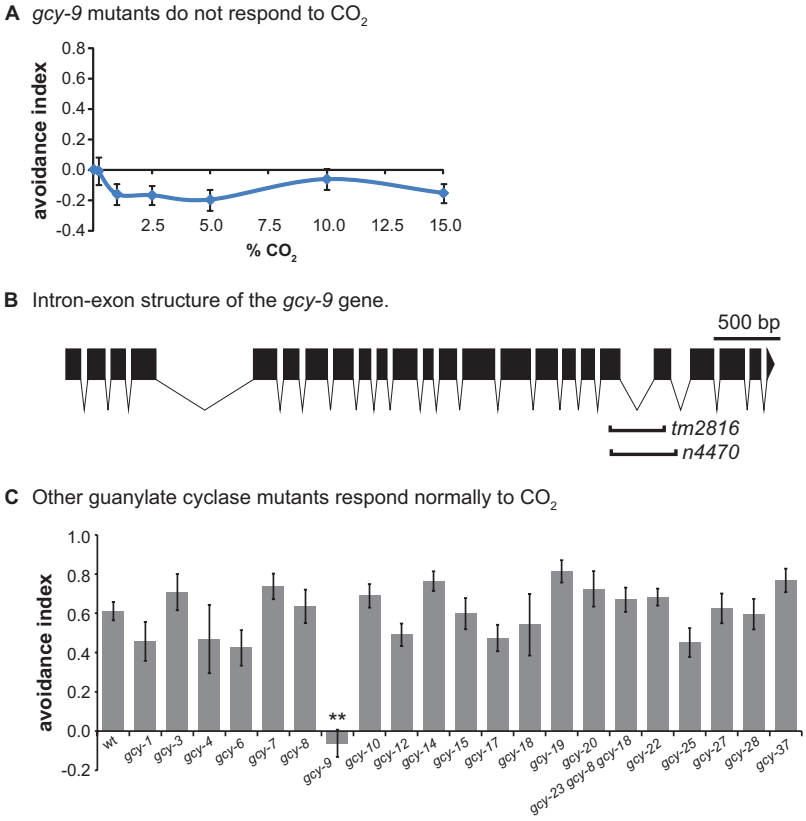

Fig. S8. *gcy-9* mutants do not respond to CO_2. (*A*) *gcy-9* mutants do not respond to CO_2 across a wide range of concentrations. (*B*) The intron–exon structure of the *gcy-9* gene. The top bracket indicates the *tm2816* deletion; the lower bracket indicates the *n4470* deletion. (*C*) *gcy-9* mutants do not respond to CO_2, whereas other receptor guanylate cyclase mutants respond normally to CO_2 (n = 5–32 trials for each genotype; wt, wild type).

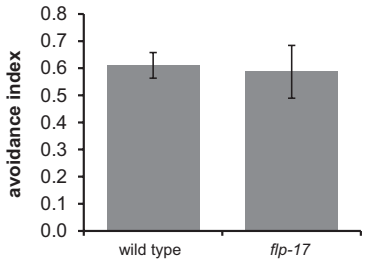

Fig. S9. *flp-17* mutants respond normally to CO_2 (n = 12–32 trials for each genotype).

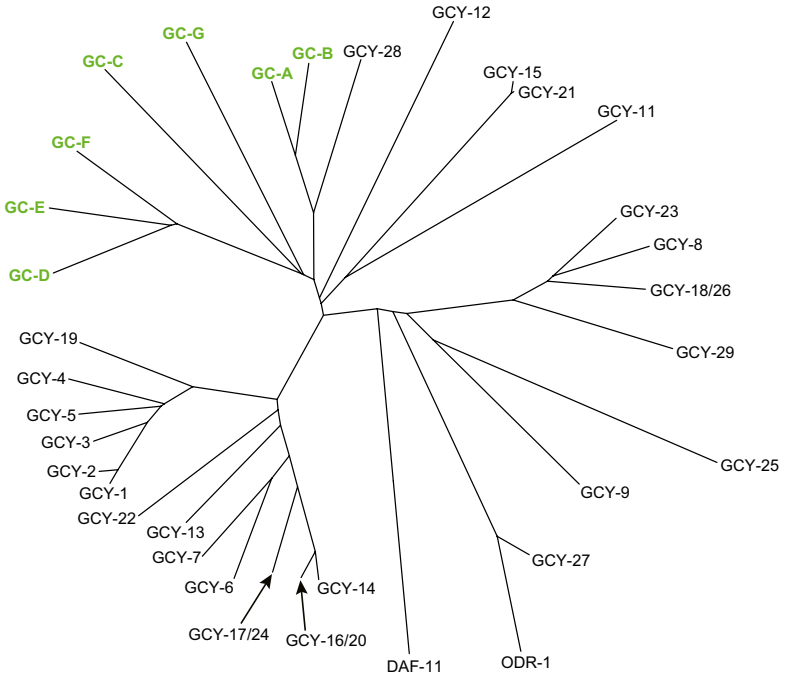

Fig. S10. The receptor guanylate cyclases of *C. elegans*. A dendrogram of receptor guanylate cyclases in *C. elegans* (1) and mammals (2). Mammalian guanylate cyclases are highlighted in green. Sequences were aligned with ClustalW. GCY-9 is part of a nematode-specific expansion of the GCY family. The guanylate cyclase family also includes seven soluble guanylate cyclases, GCY-31 to GCY-37 (1), which are not shown here.

1. Yu S, Avery L, Baude E, Garbers DL (1997) Guanylyl cyclase expression in specific sensory neurons: A new family of chemosensory receptors. *Proc Natl Acad Sci USA* 94:3384–3387.
2. Biswas KH, Shenoy AR, Dutta A, Visweswariah SS (2009) The evolution of guanylyl cyclases as multidomain proteins: Conserved features of kinase-cyclase domain fusions. *J Mol Evol* 68: 587–602.

Dataset S1. Genes that showed enriched expression in embryonic BAG neurons relative to the aggregate of all other embryonic cells

Dataset S1

Dataset S2. Genes that showed enriched expression in embryonic BAG neurons relative to the aggregate of all other embryonic cells, annotated with gene ontology (GO) terms for the biological process, molecular function, and cellular component GO categories

Dataset S2